DNA LABORATORY SET UP

A. SCOPE

The sensitivity of PCR-based analysis, involving the amplification of minute quantities of DNA, necessitates precautions to avoid contamination of samples by other sources of DNA. To minimize the potential for laboratory induced DNA contamination, three separate work areas will be used for PCR-based analysis procedures: 1) DNA extraction work area; 2) PCR setup work area; and 3) amplified DNA work area. The following section addresses the equipment, supplies, and special precautions for each area. All equipment and supplies are dedicated to each area.

B. DNA EXTRACTION WORK AREA

This work area should be used for extraction and isolation of DNA and waste disposal of unamplified extracted material. Refer to the Primary Examination Procedural Manual (documents 1590 and 1594) for procedures to be followed regarding cutting and swabbing of evidence.

Equipment and Supplies:

Pipettes
Microcentrifuge tube racks
Microcentrifuge tubes
Microcentrifuges
Spin baskets
Forceps
Sterile aerosol resistant pipette tips
Disposable gloves
Refrigerator, freezer
Heating blocks
Vortexers
Masks
Qiacubes and supplies
Qiagen extraction kits
70% ethanol
Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner
DNA Exitus
UV Crosslinker
Lab coats
Eye protection (e.g. safety glasses, face shields)
Permanent markers
Hoods
Microcons

Special Precautions:

B.1 It is important that DNA extraction of **evidence samples** be performed separately from DNA extraction of **reference samples**. This precaution will help to prevent potential cross contamination between evidence samples and reference samples.

B.2 When possible, perform DNA extraction from samples expected to contain high levels of DNA, e.g. whole blood, separately from samples expected to contain a low level of DNA, e.g. touch DNA, to minimize the potential for sample-to-sample contamination. This separation may occur by ordering the samples in the same extraction from expected low amounts of DNA to expected higher amounts of DNA. However, handle each sample as if it contains a large amount of DNA.

B.3 Use disposable gloves at all times. Change gloves frequently to avoid sample-to-sample contamination; alternatively, gloves can be cleaned with 70% ethanol or a bleach-based cleaner such as Clorox Bleach Germicidal Cleaner to prevent contamination. Where possible, remove gloves whenever exiting the work area.

B.4 Clean forceps with approximately 70% ethanol after placing a sample substrate into a spin basket.

B.5 Clean work surface with a bleach-based solution, e.g. Clorox Bleach Germicidal Cleaner, prior to and after use.

B.6 Use sterile aerosol resistant pipette tips and sterile microcentrifuge tubes.

B.7 Centrifuge all tubes before opening.

B.8 Include at least two reagent blank controls with each set of DNA extractions to check for the presence of contaminating DNA in the reagents. .

B.9 Never "blow out" the last bit of sample from a pipette. Blowing out increases the potential for aerosols. Aerosols may contaminate a sample with DNA from other samples.

B.10 A bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner, will be used to wash the exposed work areas monthly. DNA Exitus will be used to clean the QIAcubes.

B.11 Refer to the DNA Quality Manual for the treatment of Critical Reagents.

B.12 Facial masks, gloves, and lab coats must be worn when opening extraction tubes. In addition, if these tubes are opened outside of a hood, eye protection must also be worn.

B.13 Each sample that undergoes the DNA analysis process must be uniquely identified to include at a minimum the laboratory number for casework samples and the STaCS barcode number for convicted offender and arrestee samples. Amplification plates must be uniquely identified. The location of a sample on a plate must be indicated in the case package, e.g. amplification sheet or plate map.

B.14 **Qiagen kits:** the receipt date, lot #, QC date, and initials of the individual who performed the quality control testing will be recorded on each kit. Discard ATL, AL, AW1, AW2 and Proteinase K one year after kit receipt.

C. QUANTITATION / PCR SET UP WORK AREA

This is a work area used for setting up samples for quantitation / PCR setup. This work area can be separated in time from DNA extraction.

Equipment and supplies:

Pipettes
Sterile aerosol resistant pipette tips
Microcentrifuge tube racks
Disposable gloves
Microamplification tubes and 96-well plates
96-well plate bases
Optical adhesive covers
Strip caps
Adhesive seal applicators
Microcentrifuges
Vortexers
Masks
Lab coats
Eye protection (e.g. safety glasses, face shields)
Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner
UV Crosslinker
Yfiler kits
PowerPlex 16 HS kits
GlobalFiler Kits
Plexor HY kits

Special Precautions:

C.1 96 well plates should be placed in a base and not directly on the counter. This prevents the bottom of the wells from becoming contaminated.

C.2 Always add DNA to the PCR / quantitation reaction mix last. This minimizes cross-contamination by reducing the number of opportunities for inadvertent transfer of DNA.

C.3 Use disposable gloves at all times and change them frequently to avoid sample-to-sample contamination; alternatively, gloves can be cleaned with 70% ethanol or a bleach-based cleaner such as Clorox Bleach Germicidal Cleaner to prevent contamination.

C.4 Avoid touching the inside surface of the tube caps.

C.5 Change pipette tips after addition of each sample of DNA to the PCR / quantitation reaction mix.

C.6 **GlobalFiler Kit**: Store the GlobalFiler primer, master mix and positive control amplification reagents at approximately –20°C in the pre-amplification room. Once thawed, store these GlobalFiler Kit components in a rack, container or the original box at approximately 4°C in the pre-amplification room. Store the allelic ladders in the post-amplification room at approximately –20°C with the expiration date indicated on the tube or a label on the container. Once thawed, store the ladders at approximately 4°C in the post-amplification room. The lot #, expiration date, date of QC and initials of individual who performed QC will be recorded on both the boxes at approximately –20°C in the pre-amplification and post-amplification rooms and the racks, containers or boxes at approximately 4°C in the pre-amplification room.

C.7 **Yfiler Kit**: Store the DNA primer, reaction mix and positive control amplification reagents together in a rack, container or the original box labeled with: lot #, expiration date, date of QC and initials of individual who performed the QC. These racks/containers are stored in the refrigerator in the DNA extraction work area. Store the AmpliTaq Gold in the freezer with the expiration date indicated on the tube or a label on the container. Store the allelic ladders in the post-amplification room at approximately –20°C with the expiration date indicated on the tube or a label on the container.

C.8 **GeneScan-500 LIZ and GeneScan-600 LIZ Size Standards**: Store the 500 LIZ and 600 LIZ size standards in the post-amplification room refrigerator.

C.9 **Plexor HY:** Store the Plexor HY Kit at approximately –20°C in the pre-amplification room. Once thawed, store the Plexor HY Male Genomic DNA Standard at approximately 4°C in the pre-amplification room; do not refreeze.

C.10 Facial masks, gloves, and lab coats must be worn when opening extraction tubes during quantitation and PCR setup. In addition, if these tubes are opened outside of a hood, eye protection must also be worn.

C.11 Clean work surface before and after use. A bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner, will be used to wash the exposed work areas monthly.

D. AMPLIFIED DNA ROOM

This work area is a physically separate room used only for those activities that involve the handling of amplified DNA. This includes DNA amplification, electrophoresis of amplified DNA, waste disposal of amplified DNA solutions, and storage of amplified DNA.

Dedicated Equipment and Supplies:

Thermal Cyclers
Pipettes
Disposable gloves
Lab coats
Eye protection (e.g. safety glasses, face shields)
Microcentrifuge tube racks
Microamplification tubes and plates
Sterile aerosol resistant pipette tips
Genetic Analyzers / CEs, software and supplies
Refrigerator and freezer
AB 7500 Real-time PCR instruments and software
Centrifuges
Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner
DNA Exitus

Special Precautions:

D.1 Always remove gloves when leaving the amplified DNA work area to avoid the transfer of amplified DNA into other work areas.

D.2 Reduce the unnecessary dispersal of DNA around the work area by changing gloves whenever they may have become contaminated with amplified DNA; alternatively, gloves can be cleaned with 70% ethanol or a bleach-based cleaner such as Clorox Bleach Germicidal Cleaner to prevent contamination.

D.3 A bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner, will be used to wash the exposed work areas monthly.

D.4 Store tubes and plates of amplified DNA in the post-amplification room's refrigerator protected from evaporation, e.g. these tubes and plates can be wrapped in parafilm. Upon case completion amplified DNA may be discarded.

D.5 DO NOT clean the 3130 or any of its accessories with ethanol or bleach-based solutions. Deionized water is sufficient. DO NOT re-use septa from sample plates.